POTENTIAL COAGULATION FACTOR CHANGES AFFECTING MALE REGULAR WHOLE BLOOD DONORS

BY

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LIST OF ABBREVIATIONS

AMI	Acute Myocardial infarction
APTT	Activated partial thromboplastin time
AT	Anti-thrombin
Ca^{2+}	Ionized calcium
CLSI	Clinical and Laboratory Standard Institute
CVD	Cardiovascular disease
FDP	Fibrin degradation product
FVIII	Factor VIII
HCII	Heparin Cofactor II
HUSM	Hospital Universiti Sains Malaysia
IHD	Ischaemic heart disease
NBC	National Blood Center
PL	Phospholipid
PPP	Platelet poor plasma
PT	Prothrombin time
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
VTE	Venous thromboembolism

VWF	Von Willebrand factor
VWF:Ag	Von Willebrand factor antigen
WB	Whole blood

POTENSI PERUBAHAN FAKTOR KOAGULASI TERHADAP PENDERMA DARAH TETAP DI KALANGAN PENDERMA DARAH LELAKI

ABSTRAK

Manfaat pendermaan darah utuh terhadap kesihatan penderma darah tetap menjadi topik perbincangan menarik kebelakangan ini. Walaubagaimanapun, kajian yang dijalankan terhadap subjek ini di Malaysia dan di tempat lain adalah terhad. Oleh itu, kajian ini dijalankan untuk menilai perubahan faktor koagulasi tertentu terhadap penderma darah utuh tetap dan dibandingkan dengan penderma darah utuh kali pertama. Profil koagulasi ini boleh meramal manfaat kesihatan terhadap penderma darah tetap. Kajian terdahulu melaporkan penurunan faktor 'Von Willebrand' (VWF) berikutan terapi bekam. Kesan yang sama mungkin dilihat pada penderma darah utuh tetap, dimana mereka mungkin dilindungi daripada mendapat penyakit kardiovaskular (CVD) yang disebabkan oleh beban hemostatik.

Satu kajian keratan rentas telah dijalankan di Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) bermula Disember 2014 sehingga April 2016; melibatkan lelaki Melayu penderma darah tetap (subjek) dan penderma kali pertama (kontrol). Penderma darah tetap didefinisikan sebagai seseorang yang menderma darah secara rutin 5 kali dalam masa 2 tahun. Sebanyak 79 sampel penderma telah dikumpulkan dan dianalisa untuk ujian 'prothrombin time' (PT) dan 'activated partial thromboplastin time' (APTT) (sebagai ujian saringan) dan 'D-dimer', 'fibrinogen', faktor 'Von Willebrand' (VWF:Ag) dan 'heparin cofactor II' (HCII). Semua data dianalisa dengan menggunakan perisian SPSS versi 23.0.

Semua penderma darah adalah berumur diantara 18 dan 54 tahun. Kami mendapati bahawa tiada perkaitan diantara umur kumpulan subjek dan kontrol (umur 50 tahun 'cut off'). Kekerapan pendermaan di kalangan penderma tetap menunjukkan 30.8% adalah menderma melebihi 20 kali (12 daripada 39 penderma).

Parameter koagulasi pada semua penderma adalah normal iaitu min (SD) adalah 2.72 (0.59), 0.28 (0.45), 107.04 (32.85), 98.20 (32.86); masing-masing bagi fibrinogen, D-dimer, HCII and vWF. Kami mendapati adanya kaitan positif antara HCII dan fibrinogen (r = 0.201 dan '*p*-value' <0.01). Nilai persentil ke-99 bagi HCII dan fibrinogen di kalangan penderma tetap adalah rendah berbanding penderma kali pertama. Bagi VWF:Ag, nilai persentil ke-99 untuk kumpulan darah selain O bagi penderma tetap lebih rendah berbanding penderma kali pertama (masing-masing 196.6% dan 210.2%).

Walaupun kajian ini menunjukkan tiada perkaitan bagi min faktor koagulasi di antara penderma darah tetap dan kali pertama, tetapi nilai persentil ke-99 menunjukkan corak penurunan faktor koagulasi di kalangan penderma tetap. Penemuan ini mungkin menunjukkan beban hemostatik lebih rendah dikalangan penderma tetap, yang menunjukkan kelebihan manfaat kesihatan kepada mereka.

POTENTIAL COAGULATION FACTOR CHANGES AFFECTING MALE REGULAR WHOLE BLOOD DONORS

ABSTRACT

The potential health advantages of regular whole blood (WB) donation have been a topic of interest recently related to WB donors. However limited studies are found in Malaysia and elsewhere on this subject. Thus, this study was done primarily to compare selected coagulation factors in male regular and first time WB donors. Assessment of coagulation profile is one way to predict the health advantage of regular donation. Previous study reported reduction in Von Willebrand factor (VWF) following cupping therapy. Similar effect may be seen in regular WB donors which confers protection from cardiovascular disease (CVD) due to haemostatic burden.

A comparative cross sectional study was conducted in Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) from December 2014 until April 2016. The participants were Malay male from regular (subject) and first time (control) WB donors. Regular WB donor was defined as a person who routinely donated blood 5 times within the last 2 years. A total of 79 samples were collected and analyzed for coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (APTT) (as screening test) and others including D-dimer, fibrinogen, Von Willebrand factor (VWF:Ag) and heparin cofactor II (HCII). All the data were analyzed using SPSS software version 23.0.

The age of all blood donors ranged between 18 and 54 years old. There was no statistical difference of age between the study and control groups (taken at 50 years old cut off age).

The frequency of donation among regular donors showed about 30.8% of them donated more than 20 times (12 out of 39 donors).

All the donors showed normal coagulation parameters with mean (SD) of 2.72 (0.59), 0.28 (0.45), 107.04 (32.85), 98.20 (32.86); for fibrinogen, D-dimer, HCII and VWF:Ag respectively. There was a significant, positive and fair correlation between HCII and fibrinogen with r=0.415 and *p*-value <0.01. The 99th percentiles cut off value for HCII and fibrinogen among the regular donors was lower than first time donors. The 99th percentiles cut off value for vWF:Ag for non-O blood group regular donors was lower than first time non-O donors (196.6% vs 210.2% respectively).

Although no statistical different between the mean of coagulation parameters among the two groups, the 99th percentiles cut off value for VWF, HCII and fibrinogen showed a reduction pattern among the regular donors. This finding probably indicate lower haemostatic burden among the regular donors, which may indicate health advantage to this group.

CHAPTER 1

GENERAL

INTRODUCTION

1.0 INTRODUCTION

Coagulation system is an important component in haemostatic system. It consists of procoagulant factors [eg: fibrinogen, factor VIII (FVIII), Von Willebrand factor (VWF)] and anticoagulant factors [eg: antiThrombin, Protein C, Protein S, Heparin Cofactor II (HCII)]. Imbalances between anticoagulant and procoagulant activities may lead to a spectrum of haemostatic disorders from bleeding tendency to thrombotic risk (Lowe *et al.*, 1997).

Specific coagulation factor deficiency causes bleeding disorders such as Haemophilia A (factor VIII deficiency), Von Willebrand disease (von Willebrand factor deficiency), factor XII deficiency and etc (Keith Gomez, 2011). While congenital deficiencies of the coagulation inhibitors, such as antithrombin, HCII, protein C and protein S have been associated with increased risk of venous thromboembolism (VTE) (Lowe *et al.*, 1997) and atherosclerosis, thus contributing to pathogenesis of cardiovascular disease (CVD) (Meade *et al.*, 1986).

To date, many types of coagulation factor abnormalities in several clinical conditions have been studied including ischaemic heart disease (IHD), stroke, cancers, and venous thromboembolism (Abdullah, 2015). Cardiovascular disease (CVD) is associated with high morbidities and mortality in this modern world. The pathology behind this includes increased in plasma levels of coagulation factors and decreased levels of coagulation inhibitors, apart from the well known conventional cardiovascular risk factors, such as hypertension, diabetic and etc. These factors can lead to activation of blood coagulation activities (Lowe *et al.*, 1997).

It is a known fact that as age increased, the clotting factors' activities will increase accordingly (Lowe *et al.*, 1997). Increased in haemostatic activity may lead to some complications such as thrombosis and acceleration of atherosclerotic process. Increased FVIII, FVII, VWF and fibrinogen levels have been shown to be independently associated with CVD (Adam *et al.*, 2009; Tanis *et al.*, 2006). Increased factor levels also indicate underlying endothelial dysfunction due to various pathological processes in the vascular system.

Hypertension is one of the examples of risk factors of CVD, and the prevalence is increasing. More than one billion people are estimated to be affected in 2025 (Zarei *et al.*, 2012). In Malaysia, the prevalence is between 14.0 to 24.1%; and the major killer in males aged 45 years and above, while in female aged 65 years and above (Yunus *et al.*, 2003). Nowadays complementary medicine has been one of the most frequently used therapies by patients with hypertension and other related diseases. One of the treatment options is cupping therapy. Previous studies found that cupping therapy significantly reduced blood pressure measurements (El Sayed *et al.*, 2013) and cholesterol levels (Saryono, 2010).

Cupping therapy shares similar principle with blood donation, in terms of removing blood from the body. The only difference between cupping therapy and blood donation is that fresh blood is removed from the vein during blood donation; while blood is removed from underneath skin in cupping therapy. However, the cupping blood is contraindicated for re-use for blood donation (Saad, 2015). One unpublished study which was done in local institution had shown changes in coagulation factors following cupping therapy. It is reasonable and plausible to study blood coagulation changes among whole blood (WB) donors based on the similar principle of blood removal effect that is expected from both methods.

Blood donation is an essential part of our healthcare system. Many potential beneficial effects could arise from regular blood donation; such as reduced risk for CVD, heart attack, stroke and other cardiovascular related morbidities. By donating the blood on regular basis; oxidative stress can be minimized thus reducing the burden of cardiovascular system. One study found that 88% risk reduction of sudden heart attack (acute myocardial infarction (AMI)) was seen among regular blood donors (Lowe *et al.*, 1997). A different study on plateletpharesis; (Nadiah *et al.*, 2013) stated that significant decreased of plasma fibrinogen, FVIII and anticoagulant proteins: protein C, protein S and antithrombin was noted after plateletpharesis. The effect however is still within normal ranges of the factors' levels; and these changes would not lead to haemostatic derangements and morbidities.

There is no previous study documented in Malaysia or elsewhere regarding coagulation factor changes in regular WB donors. On that note, this study was done to investigate possible coagulation factor changes affecting the regular WB donors by comparing with first time blood donors of same gender. Coagulation parameters that are commonly implicated with CVD were studied, which included VWF, fibrinogen, D-dimer and HCII. It is important to study the coagulation factor changes in regular WB donors and predict the potential beneficial effects; especially the long term advantage on cardiovascular related thrombotic events and atherosclerotic process. It is also beneficial to correlate procoagulant and anticoagulant factor changes among regular WB donors. At this stage, at least the preliminary beneficial effects of donation could be predicted, hence promoting blood donation as part of healthy life-style practice. Hopefully regular blood donation can be shown to reduce CVD progression or delaying the onset of illness if harmful elevation of coagulation factors is controlled by this practice.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 COAGULATION SYSTEM

2.1.1 Roles of coagulation pathway in normal haemostasis.

Haemostasis is one of a number of protective processes that has evolved in order to maintain a stable physiology. The mechanism of coagulation involves activation, adhesion, and aggregation of platelet along with deposition and maturation of fibrin. This fibrin's formation consists of intrinsic, extrinsic and common pathway (A.V. Hoffbrand 2011).

Coagulation pathway involves a series of proteins, protein cofactors, and enzymes, which interact in reactions that take place on membrane surfaces. Reactions are initiated by tissue injury and result in the formation of a fibrin clot [Figure 2.1].

This coagulation process is initiated by the interaction of the membrane bound tissue factor (TF) after vascular injury, with plasma factor VIIa. The initiation pathway is rapidly inactivated by tissue factor pathway inhibitor (TFPI) which forms a quaternary complex with VIIa, TF and Xa. Now, thrombin generation is dependent on the traditional intrinsic pathway (A.V. Hoffbrand 2011).

The intrinsic pathway of the blood coagulation cascade requires a plasma glycoprotein called factor XI. The activation of factor XI by thrombin in the presence of a negatively charged

surface, such as sulfatide, heparin, or dextran sulfate; leads to the formation of factor XIa (Davie *et al.*, 1991). In this amplification phase, the intrinsic Xase formed by IXa and VIIIa on phospholipid surface in the presence of Ca^{2+} activates sufficient Xa which then in combination with Va, PL and Ca^{2+} forms the prothrombinase complex and results in the explosive generation of thrombin which acts on fibrinogen to form the fibrin clot (A.V. Hoffbrand 2011). (Figure 2.1).

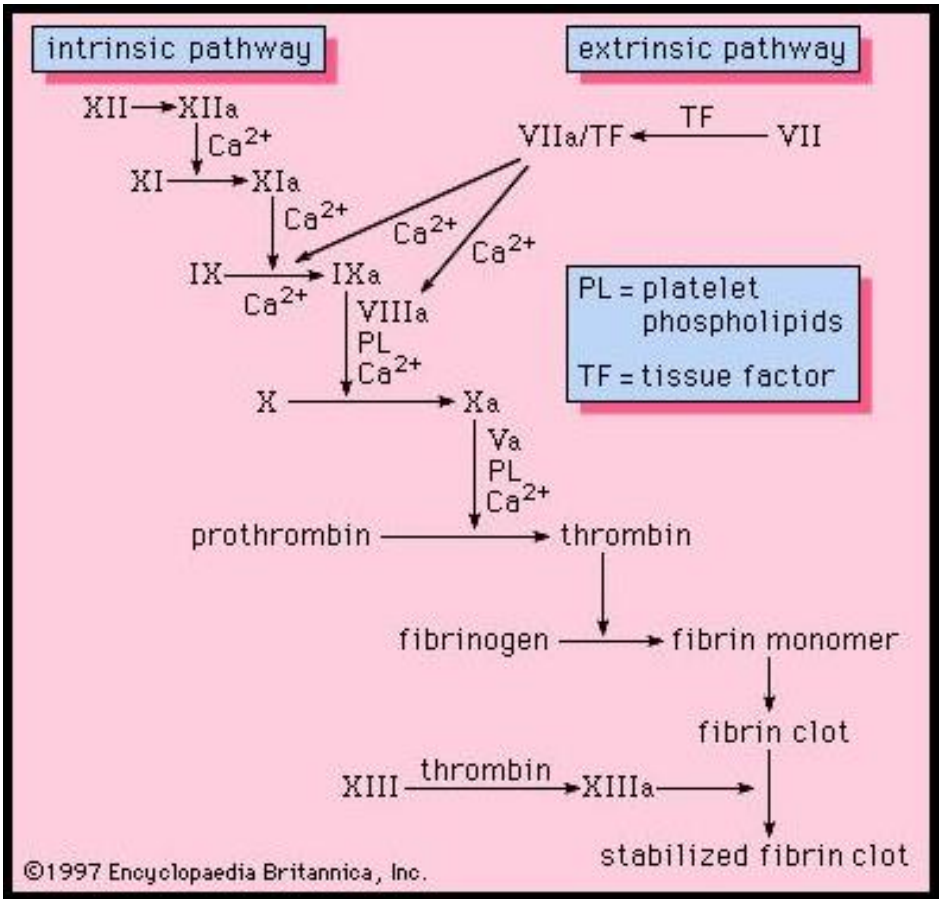

Figure 2.1:

Coagulation pathway that consists of intrinsic, extrinsic and common pathway. (Adopted from Encyclopaedia Britannica, 1997)

The normal haemostasis response to vascular damage depends on a closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors. Blood coagulation involves a biological amplification system in which relatively few initiation substances sequentially activate by proteolysis a cascade of circulating precursor proteins (the coagulation factor enzymes) which culminates in the generation of thrombin; this converts soluble plasma fibrinogen into fibrin (A.V. Hoffbrand 2011). Table 2.1 shows summary of function of specific coagulation factors.

Table 2.1: Nomenclature of the coagulation proteins/clotting factors

Clotting factor number	Clotting factor name	Function	Plasma half-life (h)	Plasma concentration (mg/L)
I	Fibrinogen	Clot formation	90	3000
II	Prothrombin	Activation of I, V, VII, VIII, XI, XIII, protein C, platelets	65	100
III	TF	Co factor of VIIa	-	-
IV	Calcium	Facilitates coagulation factor binding to phospholipids	-	-
V	Proacclerin, labile factor	Co-factor of X-prothrombinase complex	15	10
VI	Unassigned			
VII	Stable factor, proconvertin	Activates factors IX, X	5	0.5
VIII	Antihaemophilic factor A	Co-factor of IX-tenase complex	10	0.1
IX	Antihaemophilic factor B or Christmas factor	Activates X: Forms tenase complex with factor VIII	25	5
X	Stuart-Prower factor	Prothrombinase complex with factor V: Activates factor II	40	10
XI	Plasma thromboplastin antecedent	Activates factor IX	45	5
XII	Hageman factor	Activates factor XI, VII and prekallikrein		-
XIII	Fibrin-stabilising factor	Crosslinks fibrin	200	30
XIV	Prekallikerin (F Fletcher)	Serine protease zymogen	35	
XV	HMWK- (F Fitzgerald)	Co factor	150	
XVI	vWf	Binds to VIII, mediates platelet adhesion	12	10 µg/mL
XVII	Antithrombin III	Inhibits IIa, Xa, and other proteases	72	0.15-0.2 mg/mL
XVIII	Heparin cofactor II	Inhibits IIa	60	-
XIX	Protein C	Inactivates Va and VIIIa	0.4	-
XX	Protein S	Cofactor for activated protein C		-

HMWK – High molecular weight kininogen; vWf – Von Willebrand factor; TF – Tissue factor

Adopted from Indian Journal of Anaesthesia (Palta *et al.*, 2014)

In general, certain coagulation parameter is an indicator for detection of certain disease. Many types of coagulation factors in several clinical conditions have been studied including cardiovascular disease, stroke, cancers, and venous thromboembolism (Abdullah, 2015).

2.2 COAGULATION FACTORS DYSFUNCTION AND DISEASES.

2.2.1 Cardiovascular disease (CVD) .

CVD is associated with high mortality in this modern world. The mortality is associated with acute thrombotic event of the underlying atherosclerosed arteries of the heart (Abdullah, 2015).

The role of the intrinsic coagulation system on the risk of myocardial infarction is unclear. Study on plasma levels of fibrinogen and coagulation factors VII (FVII) and VIII (FVIII) was reviewed and found that these intrinsic factors were predictive of coronary heart disease (CHD). This is because the coagulation factors were deranged with increasing age. Increased in coagulation factors were greater than increases in coagulation inhibitors, especially in men. This imbalance may be related to increased activation of coagulation and hence increased risk of thrombosis, thus cardiovascular disease (Lowe *et al.*, 1997).

A cross sectional study was carried out on 59 plateletpheresis donors aged between 18 and 55 years at National Blood Center (NBC), Kuala Lumpur; compared the blood parameters before and after plateletpharesis. They found that the platelet count, FVIII, fibrinogen and thrombophilia markers anti-thrombin (AT), protein C and protein S were significantly reduced ($p< 0.05$) with prolonged PT and APTT. There were significant changes in blood coagulation parameters but it is within acceptable range. Reduction in blood coagulation parameters are compensated by humoral mechanism. This reduces risk of thrombosis. Therefore, plateletpheresis is a safe procedure for healthy donors (Nadiah *et al.*, 2013). The

same effect is probably seen in regular whole blood donor, and proof that the blood donation is safe.

Rosendaal *et al* in 2006 reported that both FVIII and FIX increased the risk of myocardial infarction (MI) among men. They found that FVIII increased the risk of myocardial infarction about 1.4-fold or more, although not dose dependent (Doggen *et al.*, 2006). Another study done among young women; Risk of Arterial Thrombosis In relation with Oral Contraceptive use (RATIO) study, had shown that FVIII clearly increased among young women. They concluded that non-O blood group, high VWF, FVIII and FIX levels were associated with an increased risk of MI in young women (Tanis *et al.*, 2006).

However, a benchmark research study published in 1998 in the *American Journal of Epidemiology* demonstrated that 88% reduced risk of sudden heart attack (acute myocardial infarction, or AMI) in regular blood donors (Lowe *et al.*, 1997). Results published in the same year demonstrated that blood donation reduced systolic and diastolic blood viscosity values from their baselines by 21% and 32%, respectively. The risk for myocardial infarction is reduced by controlling the blood pressure (Muravyov, A., et al. 2002).

2.2.1a Hypertension and CVD in Malaysia.

Cardiovascular disease (CVDs) is an important cause of worldwide preventable morbidity and mortality. The most established independent risk factors of CVDs in adults are hypertension and smoking. These factors as well as others such as hypercholesterolaemia, obesity, diabetes mellitus, stress and diet are known modifiable risk factors, whereas age,

male sex and positive family history are non-modifiable risk factors (World Heart Fideration, 2015). The prevalence of hypertension in Malaysia is between 14.0 to 24.1%. From these, more than one third of premature mortality due to chronic heart disease and a greater proportion due to stroke. In Malaysia, hypertension is more commonly seen in smokers compared to non-smokers. However, this study showed no significant association between hypertension and smoking (Yunus *et al.*, 2003).

An article that studied on prevalence of CVDs risk factors, done in Cheras Health Centre, Selangor reviewed that majority of respondents were found to be overweight or obese, two fifths had hypercholesterolaemia and a third had hypertension. The prevalence of four of five risk factors screened, such as blood pressure, body mass index, serum total cholesterol, random blood sugar and smoking status; was highest among the Malay middle aged men and lowest among the Chinese (Amplavanar *et al.*, 2010).

Another study showed significant association between hypertension and CVDs, regardless of differences in age, ethnicity, definition of hypertension and CVDs. The lowest prevalence of hypertension was seen in the youngest age groups of 15-19 years and 20-29 years. Hypertension was most prevalent in respondents aged 50-59 years and 60 years and above (Yunus *et al.*, 2003). In another study, age of onset of diabetes in population from Public Hospital in Malaysia is 44.9 +/- 12.0 (mean +/- SD) (Mafauzy, 2006).

2.2.2 Stroke.

A study on risk factors for cardiovascular disease, data on plasma levels of coagulation factors, blood pressure, serum cholesterol, and smoking were collected in 792 men 54 years

of age. During 13.5 years of follow-up, they reported that myocardial infarction occurred in 92 men, stroke in 37, and death from causes other than myocardial infarction or stroke in 60. The blood pressure, degree of smoking, serum cholesterol, and fibrinogen level measured at the base-line examination proved to be significant risk factors for infarction by univariate analyses during follow-up, and blood pressure and fibrinogen were risk factors for stroke. Fibrinogen and smoking were strongly related to each other. They found that the relation between fibrinogen and infarction, and between fibrinogen and stroke, became weaker when blood pressure, serum cholesterol, and smoking habits were taken into account, but was still significant for stroke. It is possible that the fibrinogen level plays an important part in the development of stroke and myocardial infarction (Wilhelmsen *et al.*, 1984).

2.2.3 Malignancy.

Cancer patients are associated with various morbidities including organ failure, thromboembolism, infection and etc. There is a close relationship between malignancy and haemostatic system. This includes increased procoagulant factors, anticoagulation imbalances and abnormal fibrinolysis. Venous thromboembolism (VTE) is a common problem in cancer patients but the incident varies with different types of malignancies (Blann and Dunmore, 2011). Many types of cancers are associated with VTE, including breast, malignant lymphoma, prostate, pancreas, and stomach cancer. D-dimer has been used as biomarkers to aid in the diagnosis of VTE.

2.3 COAGULATION FACTORS AND OTHER CLINICAL IMPLICATIONS.

Von Willebrand factor (VWF) is a multimeric glycoprotein that plays an important role in haemostasis. VWF serves two unique functions in haemostasis: as a carrier for FVIII and as the ligand mediating binding between platelets and the subendothelium [Figure 2.2]. Failure of FVIII to bind VWF, as occurs in type 2N von Willebrand disease, leads to its rapid turnover and resulting FVIII deficiency. VWF is synthesized by endothelial cells, megakaryocytes and platelets. In endothelial cells VWF may be secreted directly into the circulation or stored in Weibel – Palade bodies. The VWF produced in megakaryocytes and platelets is not secreted but stored in α - granules. Release of VWF from these stores occurs following activation of endothelial cells or platelets. Some therapeutic products, such as desmopressin, may work by stimulating release of stored VWF (Keith Gomez, 2011).

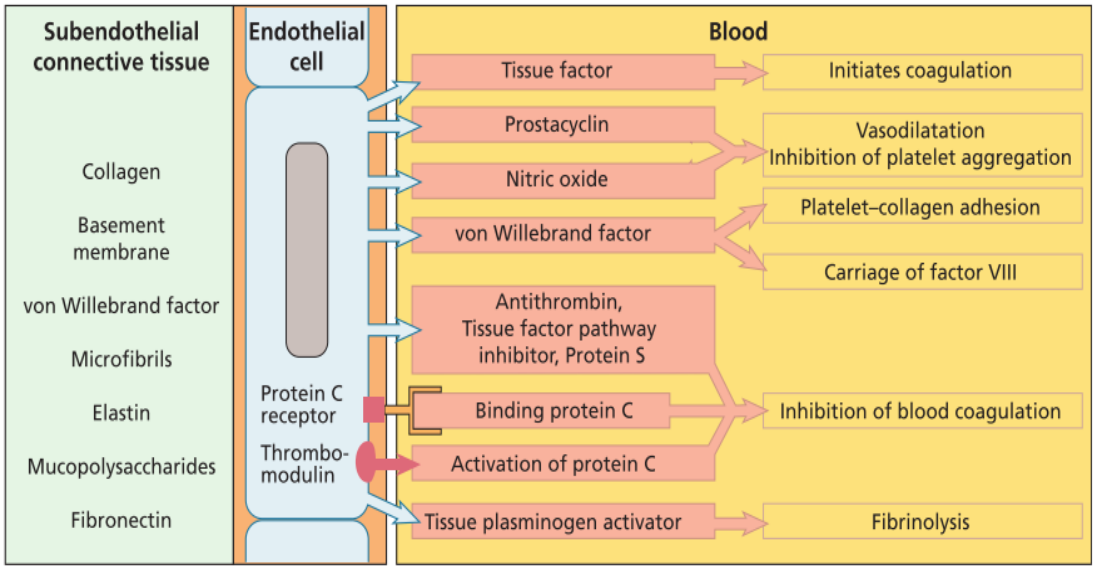

Figure 2.2:

The endothelial cell forms a barrier between platelet and plasma clotting factors and the subendothelial connective tissues. Endothelial cells produce substances that can initiate coagulation, cause vasodilatation, inhibit platelet aggregation or haemostasis, or activate fibrinolysis. Adopted from Essential Haematology, 2011(A.V. Hoffbrand 2011)

Factor VIII (FVIII) is an essential blood-clotting protein. This protein circulates in the bloodstream in an inactive form, bound to von Willebrand factor (VWF), until an injury that damages blood vessels occurs. In response to injury, coagulation FVIII is activated and separates from VWF. The active protein (FVIIIa) interacts with factor IX. This interaction sets off a chain of additional chemical reactions that form a blood clot. Defects in this gene will results in hemophilia A, a recessive X-linked coagulation disorder. FVIII is produced in liver sinusoidal cells and endothelial cells outside of the liver throughout the body. In the other hand, people with high levels of FVIII are at increased risk for deep vein thrombosis and pulmonary embolism. Copper is a required cofactor for FVIII and copper deficiency is known to decrease levels of FVIII (Wakabayashi *et al.*, 2001).

Heparin cofactor II (HCII) is present in plasma at the high concentration of 90 mg/L (1.2 μ mol/L). It appears to be a specific 1: 1 inhibitor of thrombin and to have little or no anti - FXa activity. The rate of thrombin neutralization by HCII is increased approximately 1000 - fold by heparin, although because of its lower heparin affinity it requires five to ten times more heparin than does AntiThrombin (AT). HCII has some physiological significance which is suggested by the fact that it falls in parallel with AT in DIC. However, as AT is in twofold molar excess over HCII, the latter cannot altogether compensate for a deficiency of AT, which, as stated above, is a well - established cause of a thrombotic tendency. Whether HCII deficiency leads to a similar clinical picture remains to be established, as few cases have yet been described and only occasionally have concomitant thrombotic disease been present. Mice with HCII knockout develop normally and do not show spontaneous thrombosis; however, they show an enhanced propensity to carotid occlusion after deliberate injury to the endothelium, corrected by infusion of purified HCII, suggesting that HCII has a role in prevention of arterial thrombosis (Keith Gomez, 2011).

Fibrinogen is a glycoprotein that helps in the formation of blood clots. The fibrinogen is converted by thrombin into fibrin during blood clot formation. It is synthesized in the liver by the hepatocytes. The values reported for fibrinogen, which is typically increased during acute phase and atherosclerosis, are similar to those found for other parameters (cholesterol, triglycerides, blood pressure) traditionally used for assessing cardiovascular risk. Fibrinogen concentration is correlated with cardiovascular risk factors, but modifiable lifestyle characteristics (smoking, alcohol consumption, physical activity, obesity) only explain a few percent of the total variation. Fibrinogen CV_i was also not increased in patients with underlying disease compared with healthy controls. It is noteworthy that the association with body mass index was twice as strong in women as in men (Banfi and Del Fabbro, 2009).

2.4 FACTORS AFFECTING COAGULATION TEST.

Specimen integrity is important for every laboratory test, but especially for coagulation testing, where even minor deviations from standard practices may lead to inaccurate results. Citrated plasma is the most common specimen type for routine and special coagulation testing, and the suitability of citrated plasma is particularly sensitive to anticoagulant concentration, container materials, collection technique, centrifugation, and storage (Bennett, 2015; Favaloro *et al.*, 2012).

2.4.1 Sample collection and storage.

The ideal volume ratio of blood to citrate anticoagulant is 9:1. The ratio of blood to anticoagulant is important because a relative excess of citrate, from under-filling specimen

tubes, prolongs routine coagulation test (Bennett, 2015). Prolonged APTT is nearly always observed when the tube is not filled to capacity. At a ratio of 1:7 the APTT in normal subjects is prolonged 2.5 seconds. The effect of partially filled tubes increases with longer APTT (Weston). Besides that, low plasma volume due to high hematocrit also can yield a relative citrate excess (Bennett, 2015).

In term of storage, PT is remarkably stable and no significant change in time noted after storage at 4°C for 12 hours. Plasma stored at 4°C for more than 24 hours could have shortening of the PT because factor VII is activated [Figure 2.3]. For whole blood, stored for more the 24 hours had very little change in INR, but the INR is changed sufficiently to affect anticoagulant dosage and is not recommended. At 25°C in a stoppered tube or centrifuged, the PT is stable for up to 48 hours.

APTT is more sensitive to platelet contamination and the specimen should be centrifuged if the analysis cannot be completed within 4 hours. Both PT and APTT results are reliable when platelet-poor plasma is stored at -20°C for 10 days or -70°C for 21 days. So, plasma should be platelet-poor, because freeze-thawing will break open platelets and affect the APTT results (Weston).

Effects of venous stasis during venepuncture are clinically meaningful. As hematocrit values and activities of clotting factors VII, VIII and XII are significantly increased, whereas that of activated FVII remained unchanged. Salvagno et al. hypothesized that a short-term venous stasis, as induced by up to 3 minutes tourniquet placing, might not be sufficient to produce additional procoagulant responses besides hemoconcentration (Lippi *et al.*, 2005). However,

bear in mind that venous stasis more than that will cause clotting factor activation and may cause diagnostic error in haemostasis.

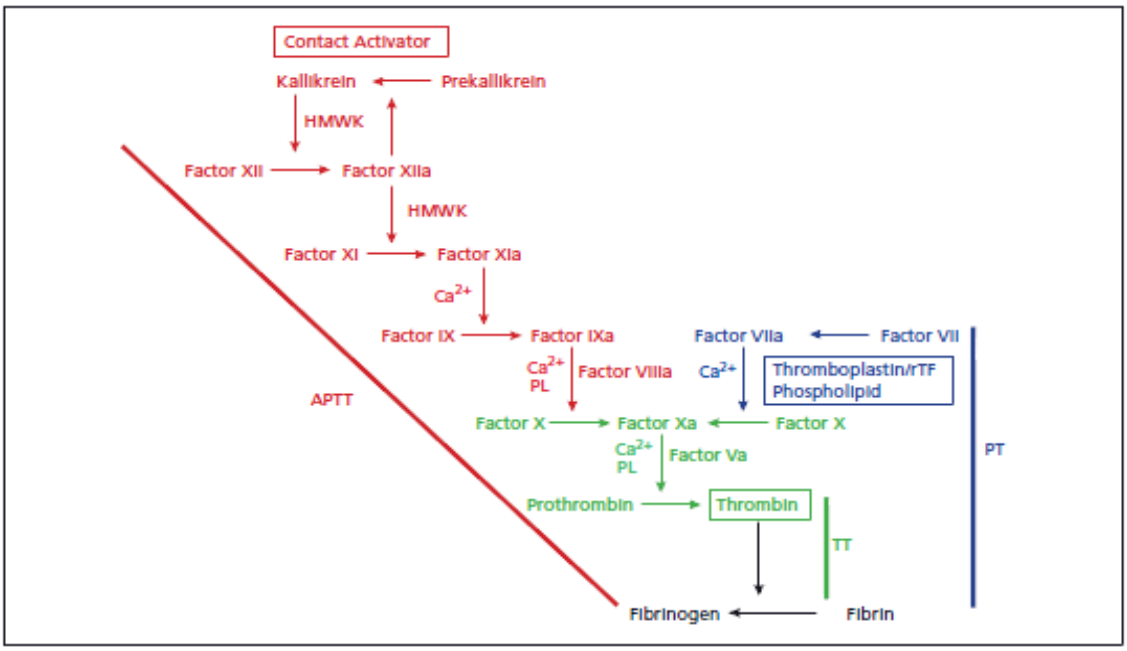

Figure 2.3:

The coagulation cascade. The traditional concept of blood coagulation with separate intrinsic (red) and extrinsic (blue) pathways converging on the common pathway (green) with the generation of FXa. The activated partial thromboplastin time (APTT) is initiated by the addition of a contact activator (e.g. kaolin or silica) and tests for deficiencies in the intrinsic pathway. The prothrombin time (PT) is initiated by addition of thromboplastin and phospholipids and tests for deficiencies in the intrinsic pathway. The thrombin time (TT) is initiated by addition of thrombin and tests for an inhibitor of thrombin (most commonly heparin) or a problem with fibrin cleavage. HMWK, high-molecular-weight kininogen; PL, phospholipid. Adopted from Postgraduate Haematology, 2011 (Keith Gomez, 2011)

2.4.2 Haemolysed sample.

Besides that, haemolysis is also an important issue to consider. Haemolysis increases the spectrometric absorbance of the plasma sample and leads to high background absorbance readings, which may compromise clot detection by some instruments and thus affect the

accuracy of test times. Cell lysis products from haemolysis include tissue factors may activate coagulation. The net effect is that detected fibrinogen levels may fall with increasing haemolysis, whereas D-dimer levels may increase. Prothrombin time values may fall in line with decreasing fibrinogen, whereas APTT may increase or decrease depending on the net effect of activation vs the loss of fibrinogen (Favaloro *et al.*, 2012).

2.4.3 Age factor.

Age, gender, ethnicity, and blood group might influence reference values for certain parameters of laboratory haemostasis, and/or generate variable test results for some tests. For example, FVIII and VWF and platelet function tests are generally influenced by such factors. Thus, interpretation of the test results should consider these factors to prevent misdiagnosis (Favaloro *et al.*, 2012).

Statistics done by Ministry of Health, merely 2.2% from Malaysian population are blood donors; compared to the developed countries in which 3.5% to 5% of their population are blood donors (BERNAMA, 2015; Hamid *et al.*, 2013).

Several studies have confirmed the age-related numerical changes in pediatric hemostasis. In general, young children have decreased physiological levels of coagulation proteins such as factors II, VII, IX, X, XI and XII, and low levels of proteins involved in fibrinolysis (plasminogen and tissue plasminogen activator) and natural coagulation inhibitors (such as antithrombin and protein C and S). However, there are no data to support an increased risk of thrombosis or bleeding during infancy. In fact it stated that neonates and children are

protected against thrombotic and bleeding complications when compared with adults (Appel *et al.*, 2012).

2.4.4 Circadian pattern.

Lower PT values observed at night, whereas a peak was recorded in the afternoon. APTT showed a peak in the evening or at night, although the magnitude of variation was small (< 10%). A nocturnal peak was reported for thrombin time, whereas a morning peak and nocturnal lower levels have been described for fibrinogen. A morning peak and a trough in the evening or at night have been described for FVIII activity (Banfi and Del Fabbro, 2009). Fibrinogen concentrations exhibited a significant high amplitude yearly variation (peak-trough is close to 28%). Peak values were observed in the period February–June and in December, whereas lower values occurred in January and the period August–September (Maes *et al.*, 1995). In diabetic patients, the peak of VWF was found in the morning (Banfi and Del Fabbro, 2009).

2.4.5 Physical activity.

Excess physical activity in patients immediately prior to collection leads to certain in vivo events (eg, plasma volume expansion and increased basal metabolism), which in turn may lead to significant effects on haemostasis. However, perhaps the best-known acute effects are related to acute phase reactants, which may rise due to physical activity, illness or stress, and include fibrinogen, VWF, and FVIII. These elevations may result in a misdiagnosis of mild hemophilia A or VWD Type 1 patients as a non-hemophilia or non-VWD; a false negative result. Blood collection may sometimes be stressful for some patients (particularly children)

leading to acute phase changes in proteins secondary to the phlebotomy itself (Favaloro *et al.*, 2012).

D-dimer is a fibrin degradation product (FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It contains two crosslinked D fragments of the fibrin protein. D-dimer concentration may be determined by a blood test to help to diagnose thrombosis. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes (Adam *et al.*, 2009). Many factors are associated with a positive D-dimer test. Age, surgery, immobility, and pregnancy are all strongly associated with D-dimer positivity. Clinicians should consider these associations when they weigh the usefulness of D-dimer testing for patients with suspected pulmonary embolism (Kabrhel *et al.*, 2010). In addition, it is used in the diagnosis of the blood disorder disseminated intravascular coagulation (Adam *et al.*, 2009).

2.5 COAGULATION FACTORS CHANGES IN RELATION TO ABO BLOOD GROUPS.

ABO histo-blood group is a major determinant of plasma levels of factor VIII (FVIII) and von Willebrand factor (VWF). They found that blood group O individuals have significantly (approximately 25%) lower plasma levels of both glycoproteins; and effect of ABO group on plasma levels of FVIII±vWF levels is primarily mediated through an effect on VWF:Ag levels. This association is of clinical significance (O'Donnell and Laffan, 2001).

Low plasma levels of either FVIII or VWF have long been established as causes of excess bleeding. Conversely, elevated FVIII±VWF levels may represent an important risk factor for

ischaemic heart disease and venous thromboembolic disease. Although with well documented association between ABO blood group and FVIII±VWF levels, the underlying mechanism remains unknown (O'Donnell and Laffan, 2001).

Theoretically, the rate of VWF synthesis or secretion within endothelial cells may be altered by ABO blood group. Alternatively, ABO group may affect VWF plasma clearance rates. ABH antigenic determinants have been identified on the N-linked oligosaccharide chains of circulating VWF and FVIII, according to the blood group of the individual (O'Donnell and Laffan, 2001). It remains unclear whether these carbohydrate structures are responsible for mediating the effect of ABO blood group on plasma VWF levels.

Plasma levels of FVIII±VWF complex are approximately 25% higher in non-O individuals than group O individuals (Koster *et al.*, 1995). An association between ABO blood group and risk of ischaemic heart disease and peripheral vascular disease has been recognized (Koster *et al.*, 1995; Meade *et al.*, 1994). This excess risk has been attributed in part to their increased levels of the plasma coagulation proteins FVIII and VWF.

Besides of ABO blood grouping, a number of environmental factors can also influence plasma VWF±FVIII levels. FVIII and VWF levels are increase with increasing age, and are higher in women than in men. Furthermore, any cause of an acute phase response (e.g. malignancy, infection or inflammation) can significantly increase the plasma levels of both FVIII and VWF. Chronically elevated VWF levels have also been described in a number of vascular disorders, including Wegner's granulomatosus and diabetes (O'Donnell and Laffan, 2001).

2.6 BLOOD DONATION.

2.6.1 Definition:

Whole blood donation is defined as blood taken from a suitable donor using a pyrogen-free anticoagulant container. The major use of whole blood is a source material for blood component preparation (National Blood Centre, 2008).

First time donor is a person who never donates blood before, that fulfilled criteria for blood donation.

Regular blood donor can be defined as a women aged 16 to 50- years-old who donates 2 or more units a year; or older than 50-years-old who donates 3 or more units a year; or a men who donates 3 or more units a year (American National Red Cross). Pusat Darah Negara defines regular whole donor as person who routinely donates blood 5 times within the last 2 years (National Blood Centre, 2008).

Repeated blood donors are advantageous to the blood donation industry as they have fewer adverse reactions to donation and lower risk of transmitting disease (Beth H. Shaz, 2013).

2.6.2 Requirements for blood donations.

Blood donation is the beginning step for blood transfusion. The blood requirement is still high around the world at present. The most acceptable means to get blood is voluntary blood donation and without financial incentive. There are reports on the factors promoting and preventing repetitive blood donation. Encouraging previous donors to return is important to increase collections of donated blood (Mathew *et al.*, 2007).

Blood donors should have the following general qualifications. They should have reached the age of consent, most often 18 years old, but 17 years old is allow with guardian permission. They should be in good health, have no history of serious illness, weigh enough to allow safe donation of a 'unit' and not recognize themselves as being at risk of transmitting infection (high risk behavior). Some blood services including Malaysia, impose an arbitrary upper limit on age, commonly 65 years old (Harvey G. Klein, 2005).

Blood donation has potential medicolegal consequences. If a donor becomes ill shortly after giving blood, the illness may be attributed to blood donation. For this reason, among others, it is important to ensure that donors have no history of medical conditions such as brittle diabetes, hypertension, poorly controlled epilepsy and unstable cardiopulmonary disease that might be associated with an adverse event following phlebotomy (Harvey G. Klein, 2005).
Blood donation is not without risk. The risk of adverse events increases based on age, weight, sex, and first-time versus repeat donations. In a study of blood donors which performed 1000 post-donation reviews, 36.1% of whole blood donors had adverse events, including arm injuries (22.7% bruise, 10.0% sore arm, 1.7% hematoma, and 0.9% sensory

changes), 7.8% fatigue, 5.3% vasovagal findings, and 1.1% nausea and vomiting (Beth H. Shaz, 2013).

2.6.3 Beneficial effects on blood donation.

Many studied had shown the beneficial effects of blood donation. Uche et al in 2013 stated that regular blood donation may be protective against cardiovascular disease as reflected by significantly lower mean total cholesterol and low-density lipoprotein levels in regular blood donors than in non-donors. This is supported by the fact that regular blood donation may lower iron stores, and this in turn lowers lipid peroxidation (Uche *et al.*, 2013).

Besides that, donation of blood can cause new red blood cells formation. The new, younger cells have more deformable membranes and less of a tendency to aggregate. Aggregated red cells may induce coagulation activity, thus may lead to thrombosis. The improved circulation from a larger proportion of young cells causes less damage to the vessel wall leading to a reduction in the build-up of atherosclerotic plaque and plaque rupture; thus reducing risk for cardiovascular disease (Muravyov *et al.*, 2002).